Abstract No. yeh657

Structural Studies of Membrane Proteins and Enzymes Involved in Glycerol Metabolism

J. Yeh (Brown University)

Beamline(s): X4A

Introduction: The Gram-positive aerotolerant anaerobes of the genera *Enterococcus* and *Streptococcus,* cannot synthesize heme and therefore lack respiratory cytochromes, catalase, and other hemeproteins. These deficiencies account for the absence of both a functional electron transport chain and oxidative phosphorylation. Biochemically, these organisms have evolved unusual mechanisms to regulate the critical functions that are typically mediated by heme-proteins. Due to the absence of heme-synthesizing machinery or a functional TCA cycle, these organisms utilize unusual biochemical adaptations to efficiently abstract energy and proliferate under aerobic environments. These effects are moderated through a number of unusual flavin-dependent redox processes. Glycerol metabolism is linked to oxidative metabolism in that in the oxidation of glycerol proceeds from glycerol-3-phosphate to dihydroxyacetone phosphate with the concomitant reduction of molecular oxygen to hydrogen peroxide. The formation of hydrogen peroxide requires a means of metabolizing in the cell this potentially deleterious molecule. We are studying several enzymes in the glycerol and oxidative metabolism pathway to understand the mechanism of reduction via utilization of sulfenic acids and how protein-protein interactions modulate activities of enzymes.

Methods and Materials: Numerous crystals were taken to X4A for 3λ MAD experiments. Crystals of SeMet-GK mutants were brought to X4A and fluorescence scans were done to determine the selenium edge within the environment of the crystal and data collected at the peak, edge, and remote wavelengths. Inverse beam geometry was used, where data were collected for 20 degrees, then the inverse 20 degrees were collected until a complete dataset were obtained. All experiments and data collection were conducted at around 100 degrees K. Additionally, we characterized membrane protein dehydrogenase crystals and collected high resolution data for a native kinase.

Acknowledgments: This work was funded in part by a MOD Basil O'Conner award and Salomon Award.